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(72) Inventor: Tofani, Santi  
10010 Burolo (To) (IT)

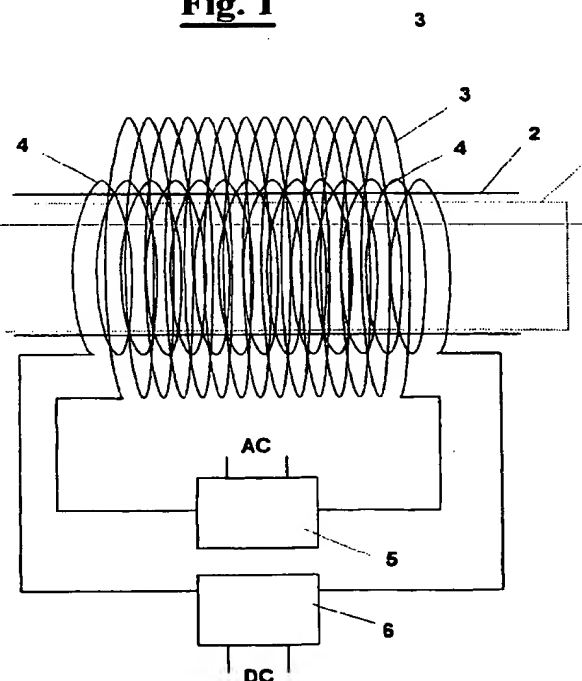
(71) Applicant: Tofani, Santi  
10010 Burolo (To) (IT)

(74) Representative:  
Celestino, Marco et al  
ABM, Agenzia Brevetti & Marchi,  
Via A. Della Spina 40  
56125 Pisa (IT)

(54) Apparatus and method for interfering with pathological cells survival

(57) A method and an apparatus for interfering with pathological cells survival, i.e. inducing apoptosis on living pathological cells, by using magnetic fields without adversely affecting normal cells. Static (S) and extremely low frequency (ELF) magnetic fields are used having intensity comprised between 1 and 30 mT. In particular SELF fields are used which are different sequences of S and/or ELF fields, i.e. S fields followed by ELF fields, ELF fields followed by S fields, S and ELF field together, as well as the presence of S or ELF fields alone, said ELF fields having a field frequency comprised between 1 and 1000 Hz. An apparatus for carrying out the method comprises means for generating static magnetic (S) fields crossing a working environment and/or means for generating electromagnetic extremely low frequency (ELF) fields over the working environment in addition to the S fields. Means are provided for modulating the S fields and varying the intensity of the S fields from 1 to 30 mT. Means may also be provided for modulating the ELF fields associated to the ELF fields generating means and imposing to the ELF fields a frequency between 1 and 1000 Hz with intensity comprised between 1 and 30 mT.

**Fig. 1**



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## Description

Field of the invention

[0001] The present invention generally relates to a apparatus for interfering with pathological cells survival.

[0002] In addition, the invention relates to a microbiological method carried out by such apparatus for interfering with pathological cells survival, in particular cells affected by cancer and other diseases caused by alterations in the mechanism of cell survival.

[0003] In particular, the interference is induced by means of static (S) and extremely low frequency electromagnetic (ELF) fields produced by the apparatus.

[0004] Magnetic Static fields and Extremely Low Frequency electromagnetic fields are hereinafter referred to also as S and ELF, respectively. Moreover, any possible combination of different sequences of S and/or ELF fields and , such as S fields followed by ELF fields, ELF fields followed by S fields, S and ELF field together, as well as the presence of S or ELF fields alone, will hereinafter be referred to also as SELF fields.

Description of the prior art

[0005] It is known that pericellular fields and currents induced by an Extremely Low Frequency (ELF) electromagnetic field, whose frequency range is from 1 Hz to 300 Hz and perhaps up to 1000 Hz induce within the cell certain membrane electrochemical events which are important for primary biologic signal transduction and amplification processes.

[0006] These biochemically mediated events then produce cytoplasmic second messengers and internal effectors such as free  $\text{Ca}^{++}$  and protein phosphorylases (kinases) which in turn trigger certain changes in the biosynthesis of macromolecules as well as carry out alterations in cellular growth differentiation and functional properties [<sup>1</sup>M. Blank, 1993].

[0007] Further, the possibility that S and ELF fields affect the DNA synthesis, DNA integrity, transcription and translation has been documented [<sup>2</sup>Liboff 1984, <sup>3</sup>Tofani 1995, <sup>4</sup>Goodman 1991, <sup>5</sup>Phillips 1992].

[0008] A possible physical mechanism to account for some of the experimental findings is the direct effect on ions (i.e.  $\text{Ca}^{++}$ ) or to ligand binding at the cell membrane [<sup>6</sup>Liboff 1985, <sup>7</sup>Chiabrera 1985, <sup>8</sup>Lednev 1991, <sup>9</sup>Blanchard 1994].

[0009] The possibility of influencing variations of  $\text{Ca}^{++}$  metabolism may lead to cell apoptosis (programmed cell death) [<sup>10</sup>Preston, <sup>11</sup>Trump 1997].

[0010] Another physical interaction mechanism is related to the possibility of influencing the kinetics of appropriate cell signalling pathways of the cell (including calcium metabolism) through a field direct effect on electron-spin motion of atoms and molecules with unpaired electrons. This influencing may affect the recombination ratio of a spin correlated free radical pair and consequently on redox signalling [<sup>12</sup>Grundler 1992; <sup>13</sup>Polk 1992; <sup>14</sup>Walleczek and Budingher 1992; <sup>15</sup>Adey 1993].

[0011] The possibility for low level, non thermal (with intensity up to 30 mT) S and ELF magnetic fields to influence in vitro the kinetics and efficacy of radical pair reactions is known from magnetochemistry [<sup>16</sup>Steiner 1989].

[0012] Naturally occurring free radicals have an oxygen- or nitrogen-based unpaired electron such as superoxide anion, hydroxyl radical and nitric oxide. These Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) can target proteins providing an obvious mechanistic explanation for free radicals-mediated signalling events. These events may influence growth factors, ion transport (i.e.  $\text{Ca}^{++}$  channels), transcription, apoptosis [<sup>17</sup>Lander 1997].

[0013] Apoptosis is a morphologically distinct form of programmed cell death that plays a major role during development, homeostasis, and in many diseases including cancer, acquired immunodeficiency syndrome, and neurodegenerative disorders. Apoptosis occurs through the activation of a cell-intrinsic suicide program. The basic genetic mechanism of apoptosis appears to be present in essentially all mammalian cells at all times, but the activation of this suicide program is regulated by many different signals that originate from both the intracellular and the extracellular environment.

[0014] Among all the genes involved in apoptosis regulation, the p53 gene is receiving much attention. This gene, which encodes a transcription factor and is common in many human cancers, mediates the cellular responses to some environmental damage. The p53 protein either can temporarily stop cell division, so that the cell can repair altered DNA, or can pilot the cell to an apoptotic death.

[0015] Published data support that p53 results in apoptosis through a three step process: 1) transcriptional induction of redox-related genes: 2) the formation of reactive oxygen species and 3) the oxidative degradation of mitochondria components, culminating in cell death [<sup>18</sup>Polyak 1997].

[0016] Moreover, published data are supporting the idea that pathological cells answer differently than normal cells to ELF fields stimuli. According to <sup>19</sup>Cadossi [1992], lymphocytes from normal patients respond differently than lymphocytes from Down's syndrome, AIDS and chronic lymphocytic leukemia patients when exposed to ELF fields (previously with mitogen).

[0017] It is also recognised that  $\text{Ca}^{++}$  influx across the membrane is influenced by ELF fields in leukemic lymphocytes but not in normal lymphocytes [<sup>20</sup>Walleczek, 1996].

[0018] With reference to chemotherapy all efforts are devoted to the target of inducing cell apoptosis in vivo instead of killing them, through Signal Transduction Directed Therapy (STDT) of cancer [<sup>21</sup>Levin, 1998].

5 [0019] Signal Transduction is an operational term that connotes the translation of genetic information into signalling cascades that allow the cell to for example interpret and respond to external stimuli and/or duplicate itself. Recent evidence suggests that alterations in the cell survival contribute to the pathogenesis of a number of human diseases, including cancer, viral infections, autoimmune diseases, neurodegenerative disorders, and AIDS. Treatments designed to specifically alter the apoptotic threshold may have the potentiality to change the natural progression of some of these

10 diseases [<sup>22</sup>Thompson, 1995].

[0020] High intensity electrical, electromagnetic and magnetic fields have been used to destroy pathological cells.

[0021] In <sup>23</sup>US4665898 an apparatus is described in which animals having malignant cells are treated by means of a high intensity pulsed magnetic field, in order to neutralize/destroy malignant cells in a selective way. This apparatus produces magnetic thermal fields having intensity comprised between 1 Tesla up to 10 Tesla and reversing polarity in the

15 range 5-1000 Kilohertz. In the preferred embodiment the magnetic field intensity is set between 1 and 50 Tesla and in particular, in the examples, it is set at 5 Tesla and 8 Kilohertz up to 18 Tesla and 250 Kilohertz.

[0022] Different ELF, thermal, continuous or pulsed fields, have been used for anti-cancer therapy in vitro [<sup>24</sup>Narita, 1997; <sup>25</sup>Rayman, 1996].

20 [0023] In these cases the fields are of very high intensity, much higher than what people are allowed to be exposed by the safety standards, and may produce heating thus damaging normal tissues and cells.

### Summary of the invention

25 [0024] It is an object of the present invention to provide a method for interfering with cell survival (i.e. inducing apoptosis) of living pathological cells (i.e. cancer cells) by using magnetic fields without adversely affecting normal cells.

[0025] It is another object of the invention to provide an apparatus for interfering with pathological cells survival.

[0026] The former and other objects are reached by the method for interfering with pathological cells survival according to the invention whose characteristic is to apply to living pathological cells (i.e. cancer cells and other diseases caused by alterations in the mechanism of cell survival) non thermal SELF magnetic fields to induce apoptosis in a

30 selective way.

[0027] For the purposes of the invention SELF fields are to be considered as different sequences of S and/or ELF fields, i.e. S fields followed by ELF fields, ELF fields followed by S fields, S and ELF field together, as well as the presence of S or ELF fields alone.

35 [0028] The concept underlying the method according to the invention is that SELF fields interfere with cell signalling sustaining cell pathological behaviour inside pathological cells, i.e. on redox signalling through free radicals, thus inducing apoptosis through a modification of p53 gene expression.

[0029] The reason why SELF fields selectively induce apoptosis in pathological cells (i.e. cancer cells) may be related to the alteration in ionic concentrations at the binding sites as well as in the corresponding enzyme activity of the pathological cell compared with the normal one.

40 [0030] For these reasons SELF fields can induce a signal programmed cell death (apoptosis), in vitro and in vivo, without causing any adverse effect.

[0031] For the same reasons SELF non thermal fields can be potentially used for treatment of cells affected by many diseases like viral infections, AIDS, autoimmune diseases, etc., where the alteration of cell survival contributes to their pathogenesis.

45 [0032] According to the invention, an apparatus for selectively interfering with pathological cells survival (i.e. inducing apoptosis in pathological cells) in vitro and in vivo has the characteristic of comprising means for generating static magnetic (S) fields crossing a working environment and means for generating electromagnetic extremely low frequency (ELF) fields in the working environment alone or in addition to the S fields.

50 [0033] Means are provided for modulating the S fields associated to the S fields generating means and varying the intensity of the S fields from 1 to 30 mT.

[0034] Means are also provided for modulating the ELF fields alone or associated to the S fields modulating means and imposing to the ELF fields a frequency between 1 and 1000 Hz with intensity comprised between 1 and 30 mT. Preferably the ELF fields have a frequency between 10 and 100 Hz. Moreover, the S and ELF fields may have an intensity variation after predetermined periods, such as in particular periods comprised between 1 to 40 minutes.

55 [0035] According to a particular aspect of the invention in the apparatus when the S fields are added to the ELF fields SELF fields are obtained with intensity ratio S/ELF comprised between 0,5 and 5.

[0036] In a particular embodiment of the invention the intensity of the S and ELF fields is set by the respective modulating means of the apparatus between 1 and 10 mT and the intensity ratio between the S fields and ELF fields is com-

prised between 0,5 and 2,5.

[0037] At least a portion of the working environment is defined by walls permeable to the S and ELF fields. At least a portion of the working environment is also advantageously adjacent to a first and a second coil respectively and the means for modulating supplying to the coils DC and AC current respectively.

[0038] Several embodiments of the apparatus are shown in the attached drawings, given as an example and are not limitative.

In figure 1 the working environment is indicated as 1 and the wall as 2. The first and second coils are given the reference numbers 3 and 4 respectively. The modulating means are diagrammatically indicated by boxes 5 and 6 respectively, and are connected to AC and DC sources.

In figure 2 a different embodiment of the apparatus, used for interfering with pathological cells survival both in vitro and in vivo has two coils 23 and 24 located coaxial to each other at the opposite sides of the working environment 21. Variable transformers 25 and 26 are provided for connected to a 50 Hz AC electric network 27. Switchable diode bridges 28 may be provided for to change the AC supply to the coils. A DC transformer 29a, a rectifier 29b as well as a timer 29c may also be provided for supplying two plates 29 so that an up to 20kV/m static (or low frequency variable up to 1000 Hz) electric field, and preferably about 6 kV/m, may be created in the working environment 21 within preferred intervals, according to the experimental conditions.

In figure 3 a further embodiment is shown of the apparatus used for interfering with pathological cells survival in vitro has a SELF modulator 35 (1-100 Hz) and two coils 33 and 34 located coaxial to each other at the opposite sides of the working environment 31. An amplifier 36 is used between the modulator 35 and the coils 33 and 34, which are supplied with the same current creating in the environment 31 either an S or an ELF magnetic field.

[0039] Another embodiment of the apparatus according to the invention (fig. 4) used for interfering with pathological cells survival both in vitro and in vivo has two Helmholtz coils 43 and 44 located coaxial to each other at the opposite sides of the working environment 41. An amplifier 46 is used between the modulator 45 and the coils 43 and 44, through a shunt element 47, which is also connected to a personal computer 49.

[0040] According to the microbiological method aspect of the invention SELF non thermal fields can be used for interfering with pathological cells survival, such as cells affected by cancer, viral infections, autoimmune diseases, neurodegenerative disorders, AIDS, etc., and are characterised by having intensity comprised between 1 and 30 mT. The SELF fields are different sequences of S and/or ELF fields, i.e. S fields followed by ELF fields, ELF fields followed by S fields, S and ELF field together, as well as the presence of S or ELF fields alone, said ELF fields having a field frequency comprised between 1 and 1000 Hz.

[0041] According to another method aspect SELF non thermal fields can be used for biotechnological genes modifications, such as in particular for modification of mutant p53 gene, and their characteristic is that said SELF non thermal fields have intensity comprised between 1 and 30 mT.

[0042] The method can be carried out alone or in combination with chemicals.

[0043] The method according to the invention will now be described in more detail by way of specific examples.

#### EXAMPLE 1

[0044] In this experiment the capability of inducing apoptosis by SELF magnetic field as a function of field intensity and frequency was studied in vitro.

[0045] Human colon adenocarcinoma cell line (WiDr) grown in confluent monolayers in T25 flasks was used for the experiment. For each exposure condition 6 flasks containing each about 10 millions cells were used, 3 exposed and 3 shame-exposed (i.e. not exposed).

[0046] During the exposure the flasks were held between two coils connected with a circuit providing DC and AC currents up to 100 Hertz. The temperature was continuously monitored and maintained at  $37 \pm 0,2$  °C.

[0047] The exposure duration was 20 minutes for each experiment and the SELF field was maintained constant. After 3 hours the cells were treated with May- Grunwald-Giemsa. Apoptosis was assessed by counting the number of apoptotic nuclei per 10 high power fields (HPF) by using an optic microscope.

[0048] The amount of induced apoptosis was evaluated by the ratio between the number of apoptotic cells found in the exposure group and the number of apoptotic cells found in the shame-exposed group, that is the group not exposed to the magnetic fields according to the invention.

[0049] Table 1 reports the results obtained in different exposure conditions.

TABLE 1

exposure conditions	SELF field composition	frequency (Hz)	field intensity (Static + ELF rms) mT	apoptosis ratio
A	S (static)	-	(0.5 + 0)	1
B	S	-	(1 + 0)	1
C	S	-	(2 + 0)	1.2
D	S	-	(3 + 0)	2
E	S	-	(4 + 0)	2,3
F	S	-	(10 + 0)	2.2
G	S	-	(20 + 0)	2.2
H	S	-	(30 + 0)	2.3
I	ELF	16	(0 + 3)	2.2
L	ELF	33	(0 + 3)	2.2
M	ELF	50	(0 + 3)	2.1
N	ELF	50	(0 + 7)	2,1
O	ELF	66	(0 + 3)	2.2
P	ELF	83	(0 + 3)	2.3
Q	ELF	100	(0 + 3)	2.1
R	S + ELF	50	(4 + 3)	2.1
S	S + ELF	50	50% of time (3 + 1) 50% of time (4,5 + 1,5)	2.2

[0050] All the results were statistically highly significant (at the t Student test). From Table 1 we can see that the apoptosis effect appears at 2 mT and doubles starting from 3 mT.

[0051] Another important finding is that apoptosis doesn't depend upon SELF field frequency. In other words during the life time of the mechanism operating the biological effect (apoptosis) the ELF field is seen as essentially constant. This means that between the two hypothesised mechanism, free- radicals (occurring in a time scale of nano- to micro-second) and ion resonance-like mechanisms, the free radical one is playing the role [26Scaiano, 1994, 27Engstrom, 1997].

## EXAMPLE 2

[0052] In this experiment the selective effect of SELF magnetic fields was verified exposing three cell lines. Two lines were malignant, human colon adenocarcinoma cells (WiDr) and human breast cancer cells (MCF-7). The normal cell line was human lung fibroblast (MRC-5).

[0053] As in the example 1 each cell line was grown in confluent monolayers in T25 flasks. The experimental protocol was the same as in example 1. Six flasks (3 exposed and three shame-exposed ) for each cell line were exposed for 20 minutes. Apoptosis was evaluated after 3 hours. The exposure conditions used were the R type of Table 1.

[0054] The results are reported in Table 2.

TABLE 2

cell line	apoptosis ratio
WiDr	2.1
MCF-7	1.4

TABLE 2 (continued)

cell line	apoptosis ratio
MRC-5	1

[0055] As shown in Table 2 only cancer cells reported an apoptosis increment statistically highly significant, whereas the normal cell line didn't. The difference in percentage of apoptosis between the two cancer cell lines was expected due to the two different duplication times. In fact WiDr duplicates faster than MCF-7. The results were evaluated at t Student test.

### EXAMPLE 3

[0056] In this example nude mice (nu/nu) bearing subcutaneous tumour masses were used to assess the influence of SELF magnetic fields on tumour growth inhibition.

[0057] Each mouse was inoculated subcutaneously with 10 million human colon adenocarcinoma cells (WiDr). Two experiments were successively carried out.

[0058] In the first experiment, 36 female mice were randomly assigned to 4 experimental groups, each formed by 6 exposed and 3 shame-exposed for a total of 24 animals exposed to 4 different SELF magnetic fields and 12 shame-exposed.

[0059] A Static Electric Field up to 6 kV/m was also applied to eventually take advantage of the different electrical behaviour between tumoral and normal tissues [<sup>28</sup>Thornton, 1984; <sup>29</sup>Barsamian, 1987]

[0060] In the second experiment 24 female mice were randomly assigned to 2 experimental groups, formed by 12 exposed to the SELF exposure condition which gave the best results among the four exposure conditions used in the previous experiment (exposure condition number 4), and 12 shame-exposed.

[0061] All the mice of both experiments were divided into experimental groups after the tumor masses for each animal were palpable.

[0062] The animals were exposed for 70 minutes, once a day, for 5 days a week, for 4 weeks. During the exposure each mouse was put in a single box made of Plexiglas held between two coils connected to a circuit providing DC and AC current up to 100 Hz respectively.

[0063] Nude mice were kept under specific pathogen free conditions and supplied with "ad libitum" diet. All the tests were conducted in accordance with the protocol issued by N.I.H. (US National Institute of Health) and N.C.I. (US National Cancer Institute).

[0064] The tumor masses were measured twice a week and their volume calculated in mm<sup>3</sup> according to the formula:

$$[(\text{major diameter}) \times (\text{minor diameter squared})] / 2.$$

[0065] After 4 weeks the animals were sacrificed and autopsied. Tumor masses were extracted, weighed and measured. Portions of tumors were used for different analysis, i.e.

- immunoistochemical: Ki-67 antigen for proliferative index, p-53 antigen for the expression of p-53 gene;
- histopathological: hematoxylin-eosin staining for the assessment of number of mitosis;
- ultrastructural: electron microscopy;
- nucleic acid hybridisation: TUNEL method for apoptosis evaluation.

[0066] In addition the following organs for each animal were extracted for histologic examination to assess the treatment toxicity: brain, heart, kidneys, liver, lungs, axillary and inguinal lymphonodes, mediastinal lymphonodes, ovaries, skin, spleen, bone marrow, subcutaneous tissue (site of tumoral cell line implantation) as well as blood tests.

[0067] The obtained results are reported in Table 3 for the first experiment and in Table 4 for the second.

TABLE 3

exposure conditions	1	2	3	4	shame-exposed
exposure duration (min)	70	70	70	70	-
time averaged field intensity (Static + ELF rms) in mT	3	3	4	6	-

TABLE 3 (continued)

exposure conditions	1	2	3	4	shame-exposed
field variation in mT (min-max) Static; [min-max] ELF	(4-6) [2-2]	(1.5-4) [1-1]	(2-5) [1.5-3.5]	(2-5) [1.5-3.5]	-
constant field time duration (min-max) in minutes	(5-15)	(5-20)	(5-15)	(5-20)	-
time % with co-presence of Static and ELF fields	0%	50%	50%	100%	-
S/ELF ratio (min-max)	-	(0,5-5)	(0,5-5)	(0,5-5)	-
time % with Static field alone	50%	50%	50%	0%	-
number of mice	6	6	6	6	12
extracted tumor mass volume (mm <sup>3</sup> )	1323 ± 304	1450 ± 288	920 ± 540	650 ± 205	1492 ± 559
extract tumor mass weight (g)	1.54 ± 0.22	1.6 ± 0.39	0.98 ± 0.56	0.96 ± 0.25	1.6 ± 0.5
number of apoptotic cells per 10 HPF	98 ± 23	115 ± 20	129 ± 25	129 ± 26	40 ± 17
p53 expression per 10 HPF	35.1 ± 0.11	43.8 ± 0.16	38.2 ± 0.06	28.7 ± 0.14	73.2 ± 0.14

TABLE 4

exposure conditions	4 (see tab. 3)	shame exposed
number of mice	12	12
extracted tumor mass volume	1139 ± 509 cm <sup>3</sup>	1914 ± 793 cm <sup>3</sup>
extracted tumor mass weight	1.4 ± 0.7 g	2.1 ± 0.6 g
apoptosis (assessed in 50% of mice only)	72.5 ± 9.3	37.0 ± 7.4
p53	35.6 ± 6.7	78.1 ± 16.7
proliferative index	0.34 ± 0.08	0.45 ± 0.07
mitosis	24.1 ± 10.9	47.7 ± 10.1

[0068] The data reported in tables 3 and 4 show that SELF fields have an inhibitory tumor growth effect in vivo. This effect, found in both experiments, was statistically highly significant (in the first experiment, mostly for the exposure condition 4) at the Dunnet and t Student tests respectively.

[0069] At the histologic examination of 12 organs for each animal for all groups no differences were found between exposed and shame-exposed mice. No differences were also found in the blood tests. These findings prove the absence of toxicity related to the SELF fields treatment.

[0070] The ultrastructural analysis by electron microscope showed in the tumor cells of exposed animals many cellular alterations: presence of apoptotic bodies and condensed cromatine near the nuclear membrane characteristic of apoptotic events.

[0071] In addition a consistent result is represented by morphological modifications, increase of number and dimensions of mitochondria as well as number of nucleoli, presence of many vacuoles inside the cytoplasm. Non neoplastic cells (i.e. epithelial and stromal cells) showed no differences between exposed and shame-exposed animals in agreement with the absence of toxicity found in 12 normal organs examined in each animal.

[0072] The increment in apoptosis as well as the decrement in p53 gene expression found in exposed mice tumors (see tables 3 and 4) are statistically highly significant (t Student test)

[0073] Results reported in Table 3 and 4 are in agreement with those obtained in vitro and shown in Tables 1 and 2.

[0074] The effect induced by the SELF magnetic fields on p53 expression enforces the apoptosis results and is in agreement with the hypothesised biophysical mechanism (i.e. free radical recombination) by which the SELF fields

have an anti-tumor effect through formation of reactive oxygen species and the degradation of mitochondrial components.

#### EXAMPLE 4

[0075] In this experiment nude mice (nu/nu) previously subcutaneous inoculated with 10 million human colon adenocarcinoma cells (WiDr) were exposed to study the animal survival.

[0076] After the cell inoculation 2 groups of mice were randomly formed respectively of 16 animals exposed and 17 shame-exposed. The mice of the former group were exposed 70 minutes once a day, for 5 days a week, for their entire life beginning after 24 hours after the tumor inoculation.

[0077] The exposure conditions were the same of the experiment the results which are reported in Table 4.

[0078] As in the previous example, the mice were maintained under specific pathogen free condition supplied with "ad libitum" diet. All the tests were conducted in accordance with protocol issued by N.I.H. and N.C.I.

[0079] The antitumor effectiveness of the treatment was evaluated by using the N.C.I. formula: ratio between exposed and shame-exposed animals of the average animal life span. This average was evaluated summing for each experimental group the time of survival divided by the number of animals. The effectiveness is obtained when the N.C.I. formula gives as result an index equal or greater than 1.25.

[0080] Table 5 reports for each experimental group, the number of living animals at different times (days) from the beginning of experiment.

TABLE 5

living mice exposed/shame-exp. (days)	16/16 (48)	16/15 (73)	15/14 (76)	14/14 (84)	13/14 (87)	12/14 (88)
living mice exposed/shame-exp. (days)	12/13 (97)	12/12 (107)	10/12 (109)	10/10 (114)	10/9 (115)	9/8 (125)
living mice exposed/shame-exp. (days)	9/7 (149)	8/6 (153)	8/5 (155)	8/4 (157)	7/4 (163)	7/3 (173)
living mice exposed/shame-exp. (days)	6/3 (183)	6/2 (192)	6/0 (194)	5/0 (195)	4/0 (203)	3/0 (257)
living mice exposed/shame-exp. (days)	2/0 (276)	1/0 (323)	0*/0			
			*sacrificed (326)			

[0081] The N.C.I. formula applied to the results reported in Table 5 gives an index equal to 1.31, that is greater than 1.25. After 194 days 6 exposed mice were alive whereas all shame exposed mice were dead.

[0082] The foregoing description of specific embodiments will so fully reveal the invention according to the conceptual point of view, so that others, by applying current knowledge, will be able to modify and/or adapt for various applications such embodiments without further research and without departing from the invention, and it is therefore to be understood that such adaptations and modifications will have to be considered as equivalent to the specific embodiments. The means and the materials to realise the different functions described herein could have a different nature without, for this reason, departing from the field of the invention. It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation.

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## Claims

1. Apparatus for selectively interfering with pathological cells survival (i.e. inducing apoptosis) in vitro and in vivo characterised in that it comprises:

- means for generating static magnetic (S) fields crossing a working environment,
- means for generating electromagnetic extremely low frequency (ELF) fields over said working environment in addition to said S fields;
- means for modulating said S fields associated to said means for generating S fields, said means for modulating said S fields varying the intensity of said S fields from 1 to 30 mT;
- means for modulating said ELF fields associated to said means for generating ELF fields, said means for modulating said ELF fields imposing to said ELF fields a frequency between 1 and 1000 Hz with intensity comprised between 1 and 30 mT.

2. Apparatus for selectively interfering with pathological cells survival (i.e. inducing apoptosis) in vitro and in vivo characterised in that it comprises:

- means for generating static magnetic (S) fields crossing a working environment,
- means for modulating said S fields associated to said generating means, said S fields modulating means varying the intensity of said S fields from 1 to 30 mT;

3. Apparatus for selectively interfering with pathological cells survival (i.e. inducing apoptosis) in vitro and in vivo characterised in that it comprises:

- means for generating electromagnetic extremely low frequency (ELF) fields over said working environment;
- means for modulating said ELF fields associated to said means for generating, said means for modulating said ELF fields imposing to said ELF fields a frequency between 1 and 1000 Hz with intensity comprised between 1 and 30 mT.

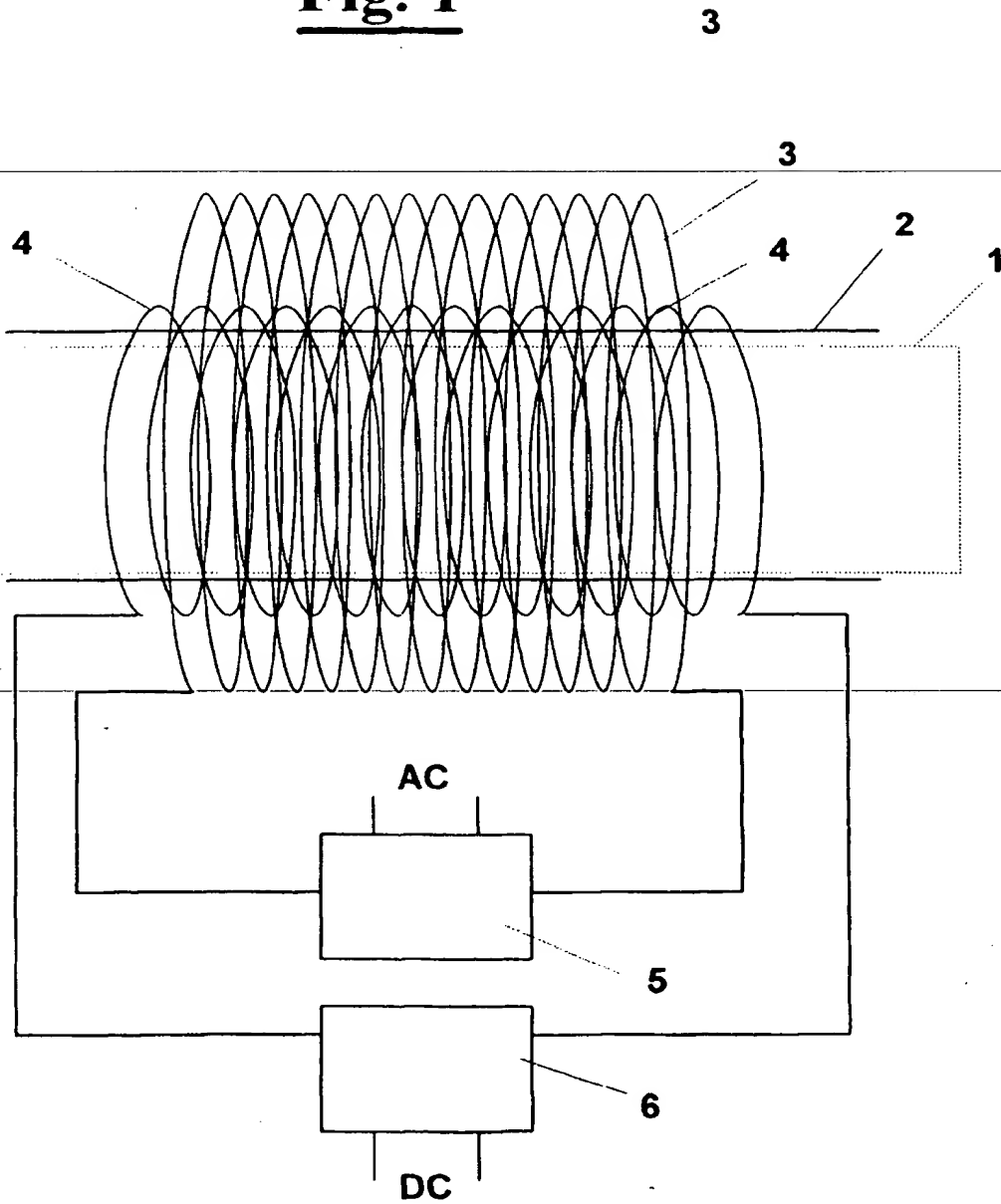
4. Apparatus according to claim 1 or 2, wherein said means for modulating said S fields cause to said S fields an intensity variation after predetermined periods.

5. Apparatus according to claims 1 or 3, wherein said means for modulating said ELF fields impose to said ELF fields a frequency between 10 and 100 Hz.

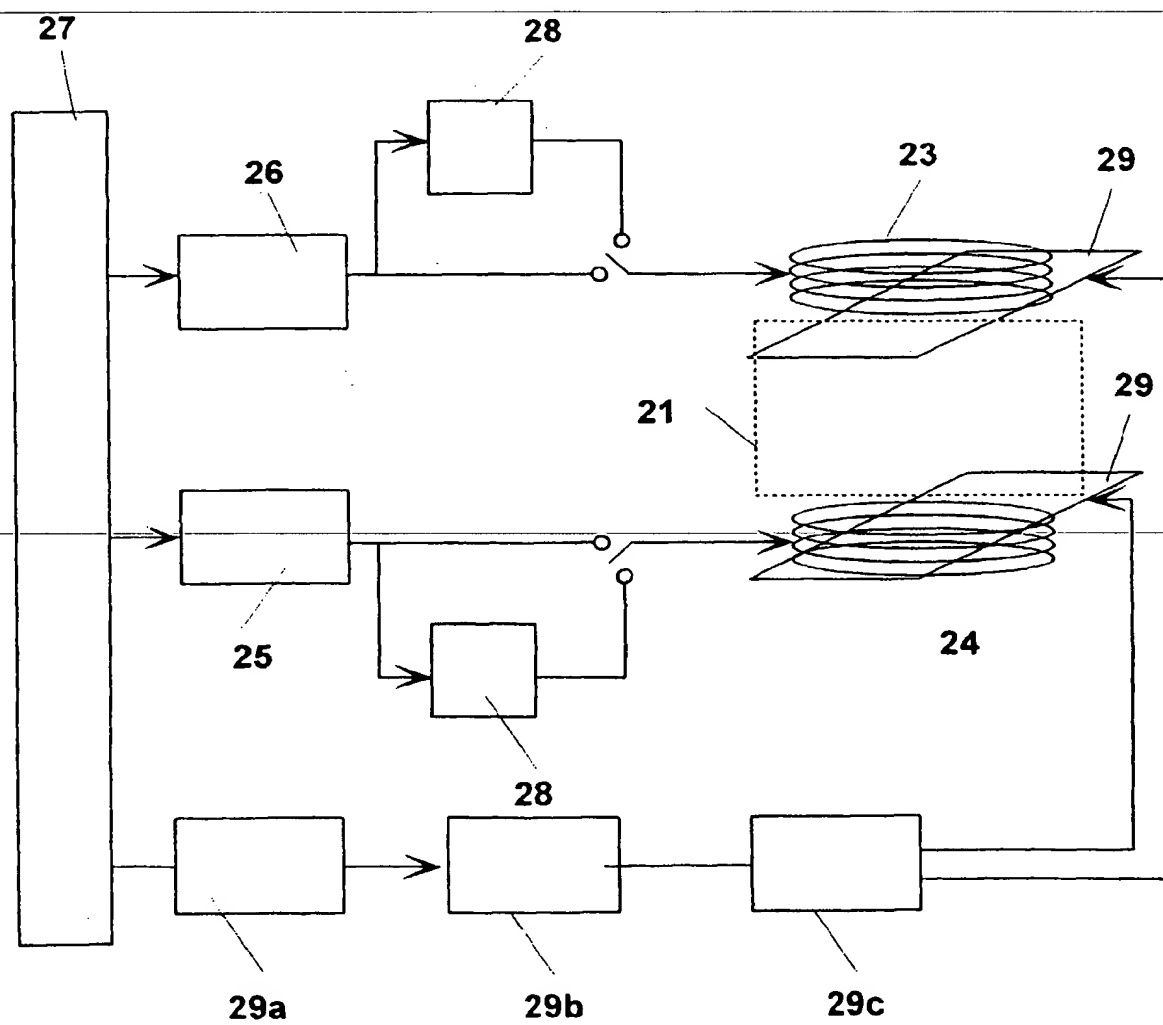
6. Apparatus according to the previous claims, wherein said S fields are added to said ELF fields obtaining SELF fields with ratio S/ELF comprised between 0,1 and 5.

7. Apparatus according to claim 6, wherein the intensity of said S and ELF fields is set by said modulating means between 1 and 10 mT and the ratio between said S fields and ELF fields is comprised between 0,5 and 2,5.
8. Apparatus according to the previous claims wherein at least a portion of said working environment is defined by walls permeable to said fields.
9. Apparatus according to the previous claims, wherein said means for generating said S and/or ELF fields comprise at least a first and a second coil respectively surrounding at least a portion of said working environment, said means for modulating providing to said coils DC and/or AC current respectively.
10. Apparatus according to the claims from 1 to 8, wherein said means for generating said S and/or ELF fields comprise at least a first and a second coil coaxial to each other, said working environment being placed between said first and a second coil and said means for modulating providing to said coils DC and/or AC current respectively.
11. Apparatus according to the previous claims, wherein means are provided for creating through said working environment a static electric field, or a low frequency variable electric field up to 1000 Hz, having intensity up to 20 kV/m.
12. The use of SELF non thermal fields for selectively interfering with pathological cells survival, such as in particular cells affected by cancer, viral infections, autoimmune diseases, neurodegenerative disorders, AIDS, etc., characterised in that said SELF non thermal fields have intensity comprised between 1 and 30 mT, said SELF fields being different sequences of S and/or ELF fields, i.e. S fields followed by ELF fields, ELF fields followed by S fields, S and ELF field together, as well as the presence of S or ELF fields alone, said ELF fields having a field frequency comprised between 1 and 1000 Hz.
13. The use of SELF non thermal fields for biotechnological genes modifications, such as in particular for modification of mutant p53 gene, characterised in that said SELF non thermal fields have intensity comprised between 1 and 30 mT, said SELF fields being different sequences of S and/or ELF fields, i.e. S fields followed by ELF fields, ELF fields followed by S fields, S and ELF field together, as well as the presence of S or ELF fields alone, said ELF fields having a field frequency comprised between 1 and 1000 Hz.
14. The use of SELF non thermal fields according to claims 12 or 13, wherein chemical substances are used in addition to the SELF fields.

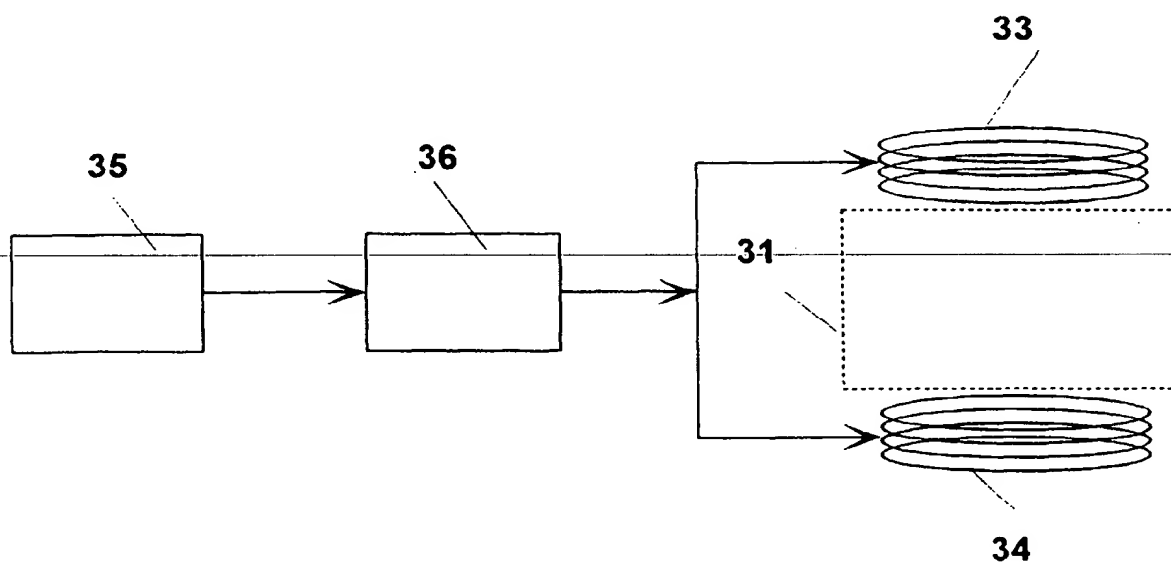
**Fig. 1**

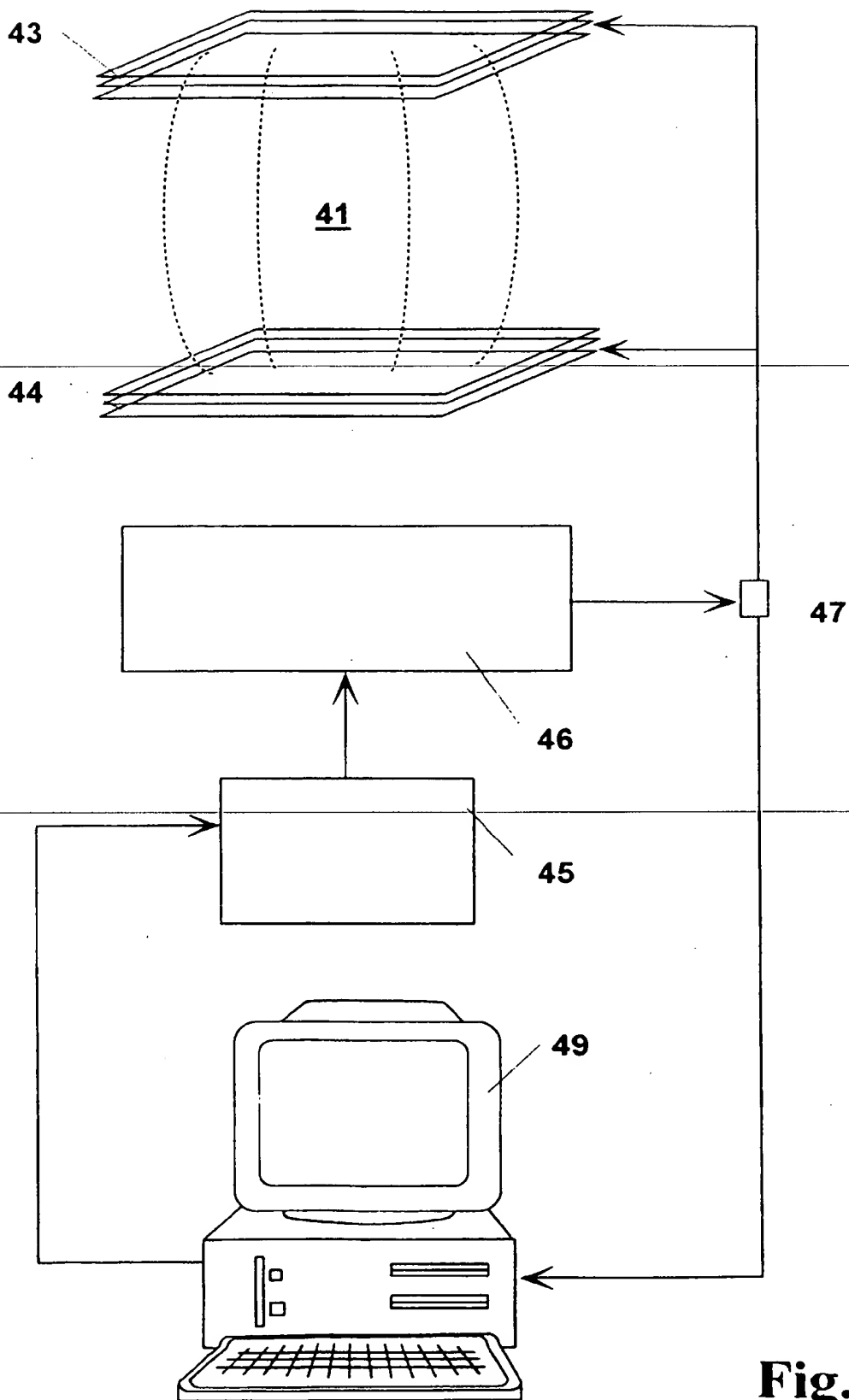


**Fig. 2**



**Fig. 3**





**Fig. 4**



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# EUROPEAN SEARCH REPORT

Application Number  
EP 98 83 0381

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X A	US 5 156 587 A (MONTONE LIBER J) 20 October 1992 * column 1, line 10-21 * * column 2, line 58-68 * * column 5, line 37 - column 6, line 16 * * column 10, line 13-20 *	3,5,9,10 1,7,12	A61N2/02
X A	DE 39 11 393 A (KRAUS WERNER) 11 October 1990 * the whole document *	3,5,9,10 1,6,7, 12,13	
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The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 11 November 1998	Examiner Grossmann, C.
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			

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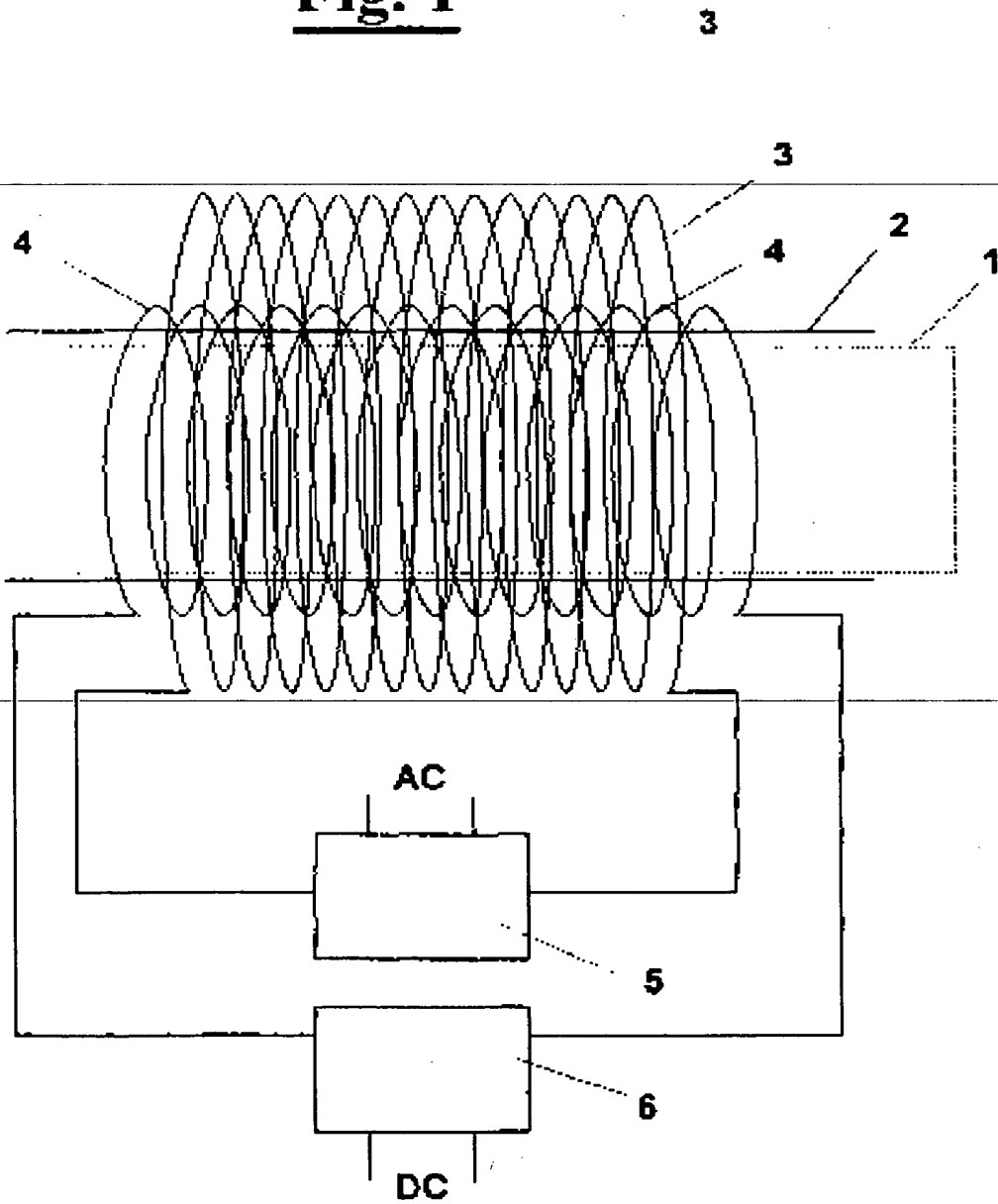
## EUROPEAN SEARCH REPORT

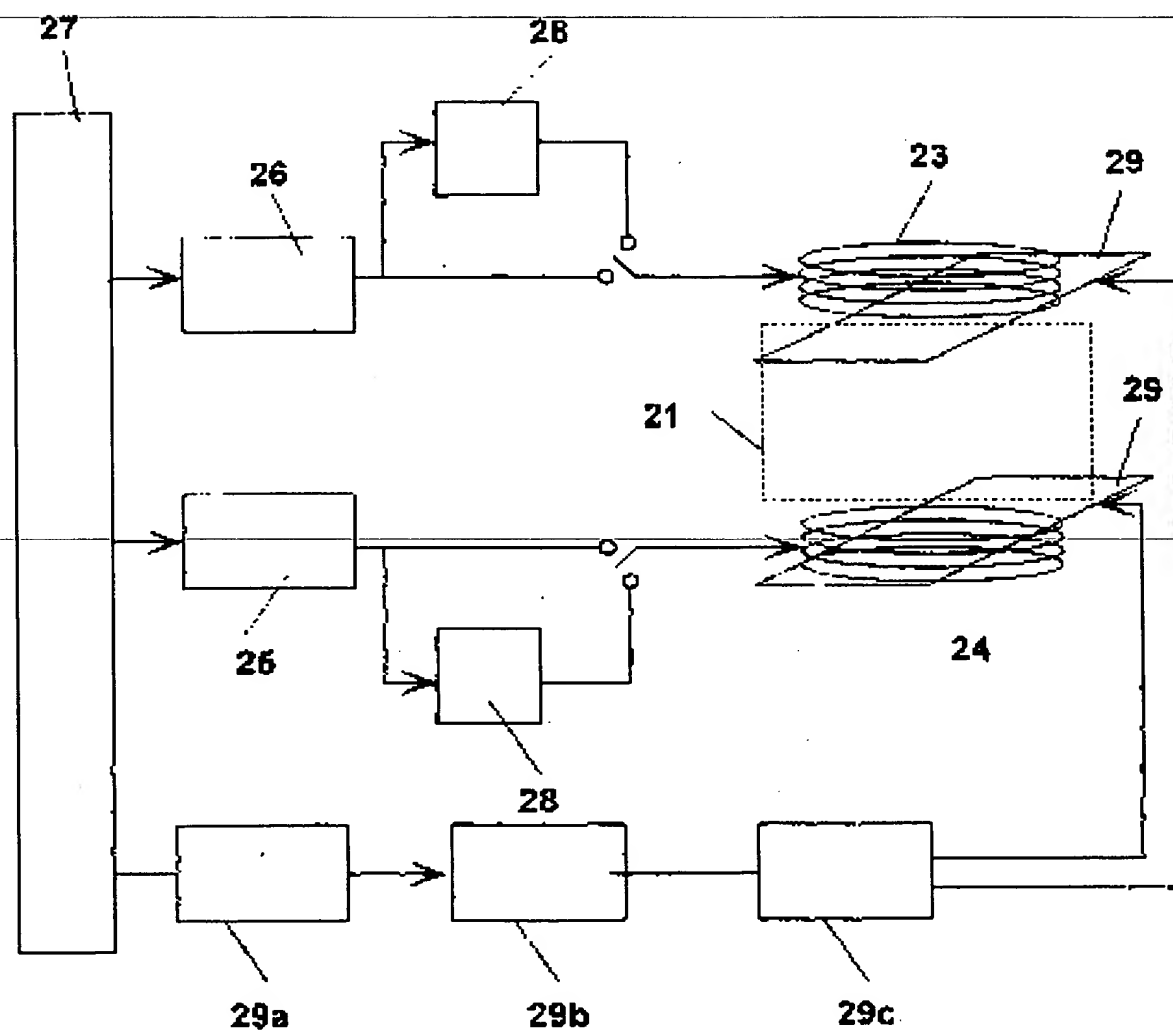
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			TECHNICAL FIELDS SEARCHED (Int.Cl.6)
The present search report has been drawn up for all claims			
Place of search	Date of completion of the search	Examiner	
THE HAGUE	11 November 1998	Grossmann, C.	
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document			

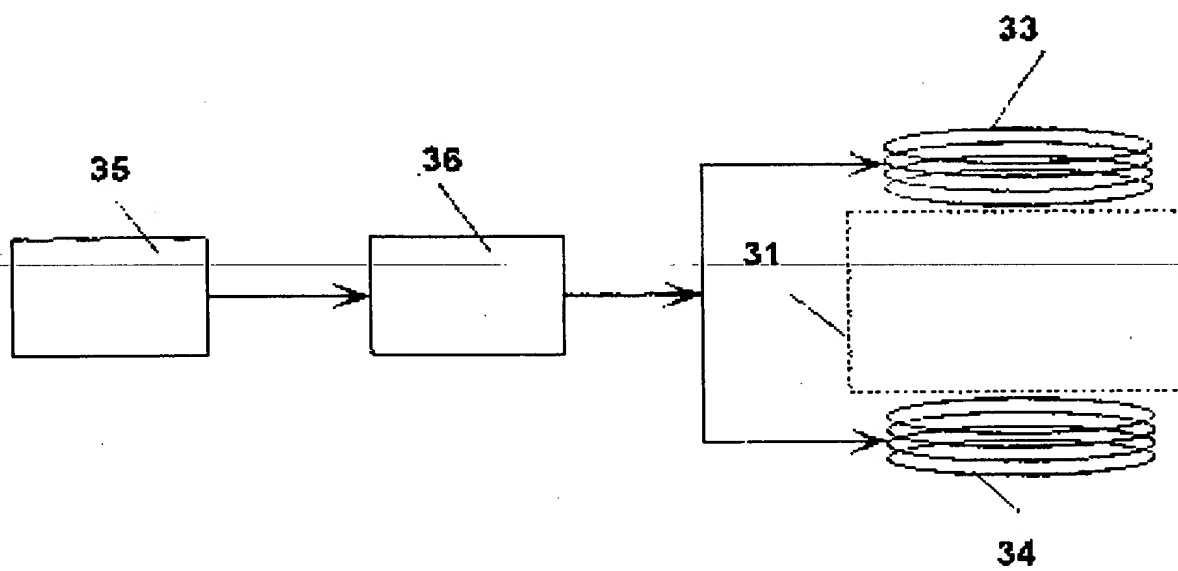
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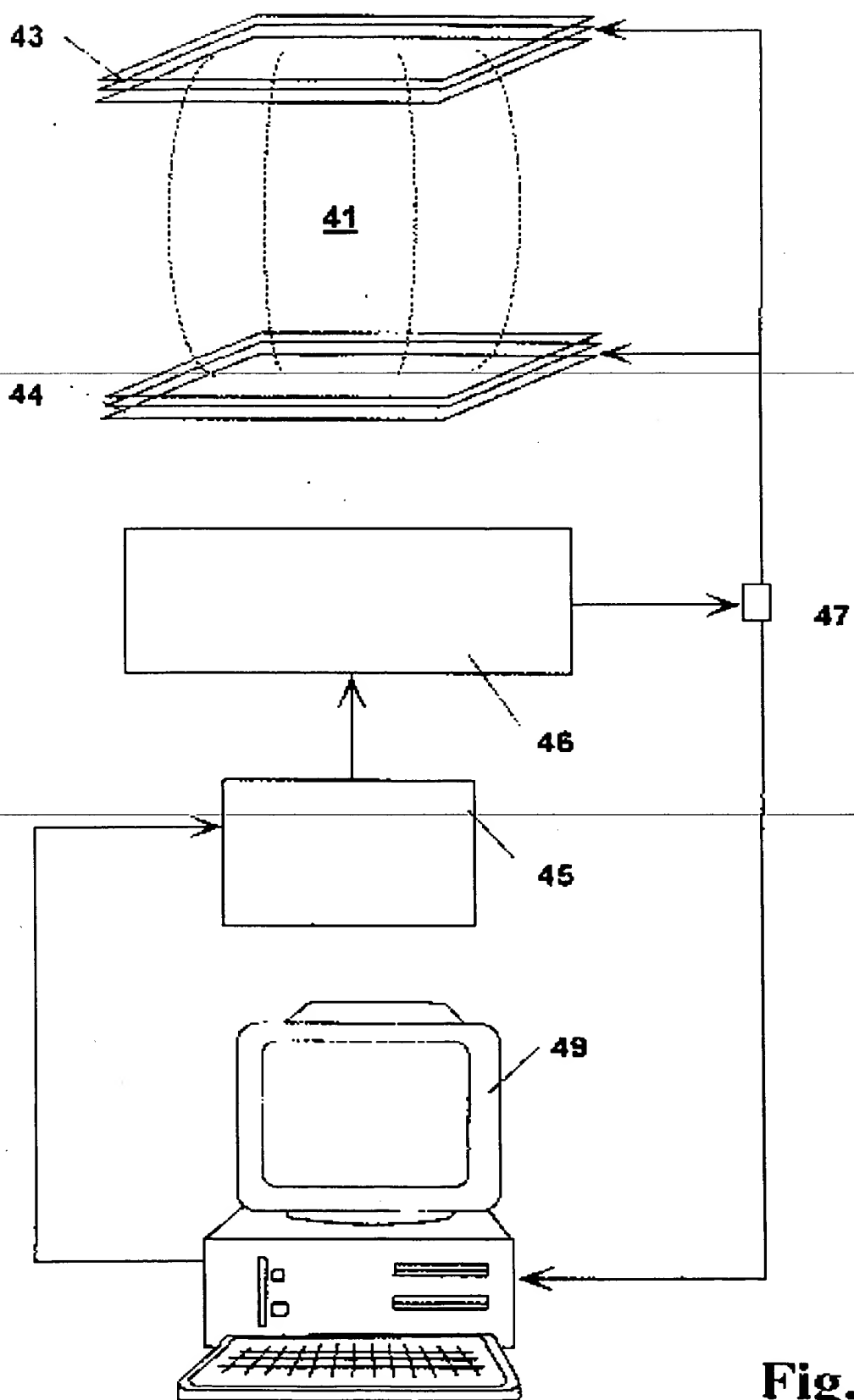
**Fig. 1**



**Fig. 2**

**Fig. 3**





**Fig. 4**